Development of a combined anaerobic and aerobic membrane bioreactor for wastewater treatment and reclamation in terrestrial-based controlled ecological life support system

Zhenmin Cheng, Yuansong Wei, Min Gao, Junya Zhang, Liangchang Zhang and Weidang Ai

ABSTRACT

A novel wastewater treatment and reuse system (WTRS) combining an anaerobic membrane bioreactor (AnMBR) and an aerobic membrane bioreactor (MBR) with the design capacity of 115 L/d was developed for a terrestrial-based controlled ecological life support system (CELSS). Results clearly showed that the WTRS realized mineralization of organic compounds and reservation of nitrogenous nutrient, therefore converting the effluent into replenishment for the hydroponic system. Trace gas emission from the WTRS could meet requirements for the whole CELSS. Compared with physico-chemical processes, the specific consumables consumption of the WTRS was advantageous but its specific energy consumption is still in need of improvement. Results of microbial community analysis were consistent with the running state of the AnMBR and the MBR.

Key words | anaerobic membrane bioreactor, controlled ecological life support system, membrane bioreactor, nitrogen, wastewater treatment

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INTRODUCTION

An environmental control and life support system is essential for manned space missions, and its most notable features are absolute tightness and limited space. Therefore, all the subsystems installed in this small airtight space must have a small footprint, high efficiency and low by-products. Long-term and multi-person space missions in the future will require a bioregenerative life support system, or controlled ecological life support system (CELSS), (Salisbury *et al.* 1997) that can achieve close to an almost complete material closure degree of 100%. Physico-chemical water treatment technologies such as those installed on the space station 'Mir' and the International Space Station (ISS) (Bobe *et al.* 2007; Grigoriev *et al.* 2011) will not be suitable any more, since they demand considerable consumables consumption. Higher plants play an important role in CELSS, and one of the most important approaches for wastewater cycles is to transform human urine into nutrient solution for higher plants (Qin *et al.* 2013). The nitrification process is generally realized in the biological wastewater treatment process. However, biological-based processes for CELSS wastewater treatment have not been applied widely. Petrie & Lupton (1991) tested a packed bed reactor treating simulating CELSS wastewater and succeeded in reducing the chemical oxygen demand (COD) concentration from 800 mg/L to approximately 100 mg/L and transforming urea into ammonia nitrogen (NH_4^+-N). Recently, a membrane

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aerated bioreactor was developed to treat wastewater from an exploration mission. Due to the low C/N ratio of the influent, removal efficiencies of organic matter and NH_4^+-N were only 83.6% and 55.6%, respectively (Meyer *et al.* 2016). Sanitary and kitchen wastewater produced in the CELSS facility 'Lunar Palace 1' was treated by a membrane-biological activated carbon reactor for preparing the nutrient solution of the plants, and 85% COD removal rate and conservation of nutrients were realized (Xie *et al.* 2017). These studies mainly focused on pollutants elimination. However, systematic research including selection of proper combined technologies, nutrients reservation and by-products such as gas emission control still needs further study.

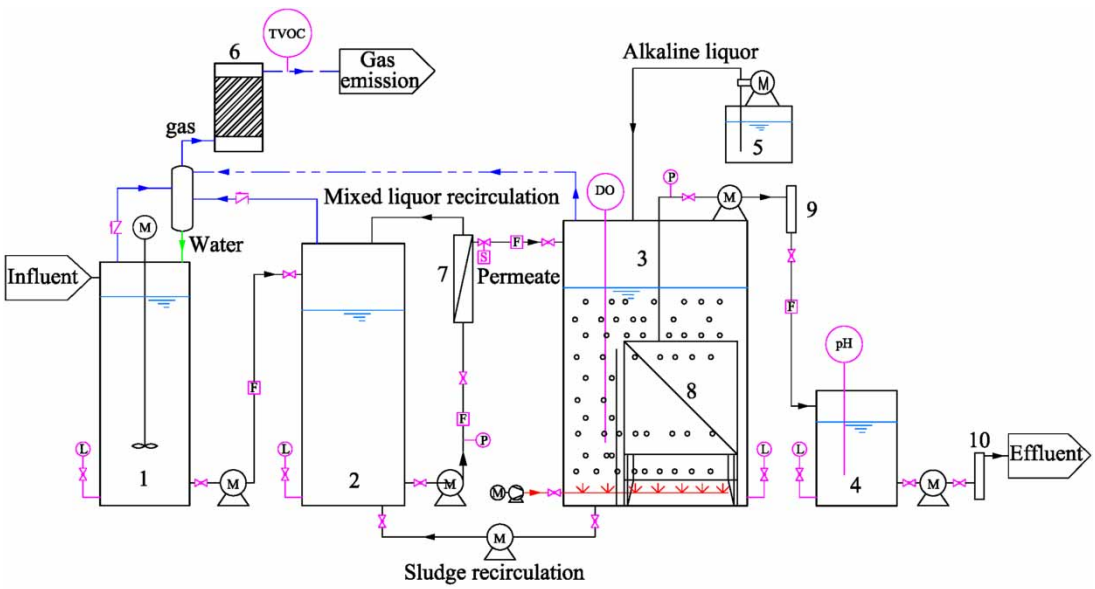
In this study, a novel wastewater treatment and reuse system (WTRS) mainly combining an anaerobic membrane bioreactor (AnMBR) and an MBR was developed and tested for a terrestrial-based CELSS, in which the effluent would be recycled to prepare the hydroponic solution with a large amount of condensate water. The performances of the WTRS on COD removal and nitrogen reservation, the characteristics of the gas emission and the evolution of a microbial community other than membrane fouling were discussed. In addition, the consumptions of power and alkaline reagent compensating for alkalinity were investigated.

MATERIALS AND METHODS

Description of the WTRS

Figure 1 is a schematic diagram of the WTRS. Its maximum treatment capacity was 115 L/d. All the tanks and reactors were tightly sealed to control gas emissions.

The buffer tank had a total volume of 120 L. The AnMBR consisted of a bioreactor tank of 61 L and two arrays of 0.03 μm, PVDF tubular membrane in parallel, each containing five modules (MO P1U(0.5 m)_I8, Tri-high). The cross-flow rate of the mixed liquor was kept above 3 m/s by a centrifugal pump (CRN 1–5, Grundfos) to control membrane fouling. The MBR was comprised of a bioreactor tank of 135 L and an immersed membrane module containing 5 pieces of 0.1 μm, PVDF flat sheet membrane (SINAP-10, SINAP). The effluent tank was equipped with two ultraviolet disinfection units (H-UV01, Sansheng). An alkaline liquor addition subsystem composed of a tank of 35 L and a diaphragm pump (P026-358TI, Milton Roy) was also installed. Gas emissions from all the tanks firstly went through a gas-water separation unit, and then were treated by two adsorption columns filled with particulate activated carbon. An automation system with PLC (Table S1 in the Supplementary data, available with the



1 Buffer tank, 2 Anaerobic bioreactor, 3 Aerobic bioreactor, 4 Effluent tank, 5 Alkaline liquor tank
6 Adsorption columns, 7 Tubular membrane modules, 8 Flat sheet membrane module, 9&10 UV disinfection units

Figure 1 | Schematic diagram of the WTRS.

online version of this paper) was developed to monitor and control the WTRS operation.

Operation and sampling

The AnMBR and MBR were seeded with 30 L and 40 L concentrated excess sludge from a municipal wastewater treatment plant (WWTP) with an A^2/O process. The WTRS was fed with synthetic wastewater composed of real urine and some detergents including toothpaste, facial cleanser, shampoo etc. The whole experimental period was divided into two stages: the adaptation stage (Stage 1, 1–35 d) and the stable stage (Stage 2, 36–84 d). The daily loading volume of wastewater and the ratio of urine in the wastewater were increased from 30 L to 100 L and 1% to 6% in Stage 1, respectively. In Stage 2, hydraulic retention times (HRTs) of the AnMBR and MBR were 10 h and 19 h, respectively. Sludge retention times of the two reactors were close to infinite, because no sludge was discharged except for sampling. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the AnMBR increased from 4.74 g/L and 3.38 g/L to 7.66 g/L and 5.02 g/L, respectively. MLSS and MLVSS in the MBR increased from 6.52 g/L and 4.26 g/L to 9.07 g/L and 7.00 g/L, respectively. The flux of the flat sheet membrane was set to 10.4 L/(m^2·h) and the filtration to idle-cleaning ratio was 4 min:1 min. The control logic of the WTRS is illustrated in Figure S1 in the Supplementary data (available online).

Samples of influent, AnMBR permeate and effluent were taken at least three times a week and kept at −4 °C before analysis. Samples of sludge were taken once a week and kept at −20 °C before analysis. Samples of gas emission at 74–84 d were collected to determine gaseous compounds and culturable bioaerosols. Two sludge samples from the AnMBR and MBR at 84 d as well as the inoculum were collected for microbial community analysis. All the data shown in figures and tables are the average value of duplicates.

Analytical methods

Water quality

MLSS and MLVSS were determined at 104 °C (4 h) and 600 °C (2 h), respectively. NH_4^+-N and nitrite nitrogen (NO_2^--N) were analyzed according to the Standard Methods 4500-NH_3-B and 4500-NO_2-B (APHA 2005). Total COD of all samples was measured using HACH 8000 methods with a DR 2800 spectrometer (HACH, USA). pH value was measured by the online sensor.

Composition of gas emission

CH_4 was determined by gas chromatography (GC, 4890D, Agilent Inc., USA) according to Yu *et al.* (2016). H_2S and NH_3 were determined using gas detecting tubes (120U and 105SD respectively, Komyokk, Japan). Total volatile organic carbon (TVOC) was measured by the online detector (AP-G-TVOC-2, Empaer). Each of values shown in figures and tables of this study is the average of duplicate samples.

Sampling for culturable bioaerosols was performed using an Andersen six-stage cascade impactor (Applied Technical Institute of Liaoyang, China) according to Xu & Yao (2013). Two antibiotic-resistant bacteria (ARB) were incubated in 20 mL nutrient agar with 16 mg/L tetracycline and 48 mg/L erythromycin, respectively. The total bacteria samples were incubated on 20 mL nutrient agar medium (LB, Ao boxing Biotech, Co., China). The two ARB and total bacteria were incubated at 28 °C for 48 h (Zhang *et al.* 2016b).

DNA extraction and microbial community analysis

4 mL of each sludge sample was pretreated and polymerase chain reaction (PCR) primers 515F/806R targeting the bacteria and archaeal 16S V4 region were selected for the microbial community structure analysis following a protocol described previously (Zhang *et al.* 2016a). The final mixtures were sent out to Sangon Co., Ltd in Shanghai for small-fragment library construction and pair-end sequencing using the Illumina MiSeq sequencing system (Illumina, USA).

Sequencing reads were assigned to each sample according to the unique 6-bp barcode. Pairs of reads from the original DNA fragments were merged using FLASH and then were filtered using QIIME quality filters. PCR chimeras were filtered out using UCHIME. The taxonomic classification of the sequences in each sample was carried out individually using RDP Classifier, and the sequences of different taxonomy levels were assigned at the bootstrap cutoff of 50%, as suggested by the RDP.

RESULTS AND DISCUSSION

COD removal and nitrogen transformation

Generally, the WTRS performance on COD removal is excellent enough to meet the designed effluent quality (<70 mg/L). As shown in Figure 2(a), in Stage 1 influent COD concentration increased from 200 mg/L to about 700 mg/L along with the rise in the urine ratio. And when the urine ratio reached the highest value, a sudden rise in effluent COD concentration was observed, which was caused by the excessive COD loading rate. The maximal value of 0.48 kg COD/(kg MLVSS·d) on day 27 was more than twice the adopted practical value (Judd & Judd 2011). Thus, on day 33, seed sludge from the same WWTP was added into the MBR. In Stage 2, the effluent COD concentration remained below 30 mg/L, with COD removal efficiency continuously above 95%. The AnMBR was designed to run under a hydrolysis-acidification state to control biogas production. So throughout the whole experimental period, HRT was quite shorter and COD removal ratio was much lower (around 40%) than those AnMBR processes (Chen *et al.* 2014; Yu *et al.* 2016) aiming at organic compounds degradation and biogas production.

Figure 2(b) shows that most of the time the NH_4^+-N concentration of the AnMBR permeate was much higher than that of the influent, revealing that urea was transformed into NH_4^+-N. Urea is a molecule that is readily metabolized by many bacteria (Thomas *et al.* 2011). Although the influent was prepared daily, when the collected urine was not fresh enough, urea hydrolyzation occurred in the buffer tank. Similar to COD, in Stage 1 the NH_4^+-N removal efficiency fluctuated along with the rise in NH_4^+-N loading rate, and during Stage 2 the WTRS exhibited excellent and stable NH_4^+-N removal ability. Besides, the NO_2^--N, which could be harmful to plants' growth, was also taken into consideration. Figure 2(c) shows that under overload of NH_4^+-N in Stage 1, effluent NO_2^--N concentration rose sharply, which partly contributed to effluent COD concentration. And during Stage 2, effluent NO_2^--N concentration was mostly below 2 mg/L. The dissolved oxygen (DO) value was consistently maintained above 2 mg/L in the MBR, so the low NH_4^+-N and NO_2^--N concentrations of the effluent indicated that full nitrification could be achieved. Nitrogen in the influent was almost completely transformed into nitrate nitrogen, achieving the designed goal.

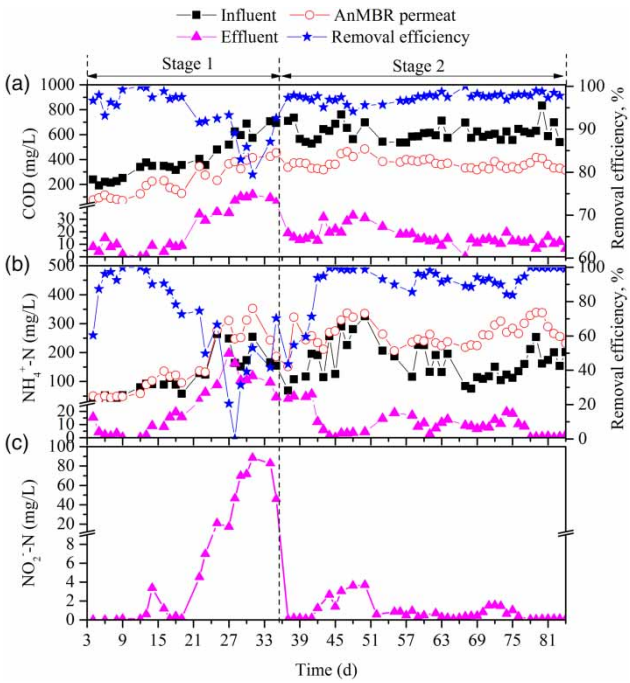


Figure 2 | Performance of the WTRS on COD removal (a), NH_4^+-N removal (b) and effluent NO_2^--N concentration (c).

Characteristics of the gas emission

In biological wastewater treatment processes, some compounds in the gas emission such as CH_4, H_2S and aerosols can be a threat to the plants and crew in tightly closed habitat. Table 1 shows that the concentrations of NH_3, H_2S,

Table 1 | Composition and concentrations of the gas emission

Day	NH_3, mg/m^3	H_2S, mg/m^3	TVOC, mg/m^3	CH_4, mg/m^3
74	<0.2	<0.1	0.2	–
75	<0.2	<0.1	0.2	–
76	<0.2	<0.1	0.2	0.54
78	<0.2	<0.1	0.25	0.54
79	<0.2	<0.1	0.27	0.3
80	<0.2	<0.1	0.26	0.49
81	<0.2	<0.1	0.27	0.61
82	<0.2	<0.1	0.24	0.55
84	<0.2	<0.1	0.21	0.13

TVOC and CH_4 were all within the threshold designed for the 4-person-180-day integrated CELSS test (0.4, 0.5, 1.2 and 2 mg/m^3 respectively) recently conducted in China.

To evaluate the possible ill-effect of the gas emission on the crew's health, detection of bioaerosols including culturable airborne bacteria, tetracycline-resistant bacteria and erythromycin-resistant bacteria were conducted at 79 d when the MLSS in the MBR was 9.07 g/L. The culturable airborne bacteria discharging rate (CABR) in the outlet was 1,783 ± 287 CFU/h, which was calculated using Equation (1).

$$\mathrm{CABR} = \frac{\mathrm{N}}{60(Q_1 t/Q_2)} \quad (1)$$

where N was the colony forming units after incubation; Q_1 was the suction rate of the vacuum pump, L/min; t was the sampling time, min; and Q_2 was the WTRS gas emission flux, L/min. Among total bacteria, the erythromycin-resistant bacteria only accounted for 2%; that is, 37 ± 7 CFU/h, whereas no culturable airborne tetracycline-resistant bacteria were detected. The seeded sludge and urine used may be the ARB sources.

Microbial community

The high coverage values of OTUs (97% similarity) (Table 2) reflected the real microorganisms profile in the inoculum and both bioreactors. The Shannon and Simpson index showed that the community diversity both declined under anaerobic and aerobic conditions.

Figure 3 shows that *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* were dominant in the AnMBR, accounting for about 80% altogether. *Bacteroidetes* and *Firmicutes* were enriched in the anaerobic sludge with proportions of 31.93% and 23.32%, which were 2.63 and 1.8 times those in the inoculum. It should be noted

Table 2 | Characteristics of microbial community diversity

Sample	Reads	OTU	Coverage	Shannon	Simpson
Inoculum	43,843	1,899	0.98	5.40	0.01
Anaerobic sludge	43,843	758	0.99	3.22	0.1
Aerobic sludge	43,843	1,240	0.99	3.75	0.1

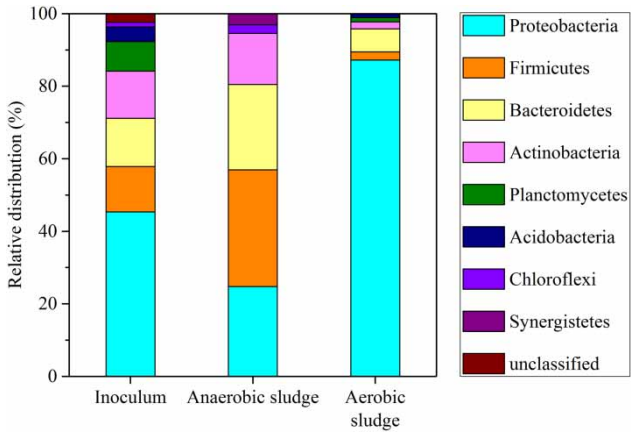


Figure 3 | Relative abundance of the major bacterial communities (>1%) at phylum level.

that the major role of the anaerobes *Bacteroidetes* in the fermentation system is to break down macromolecules such as protein, starch, cellulose and fiber (Yang *et al.* 2015). And *Firmicutes* has been reported to play critical roles at hydrolysis and acidification stages (Zheng *et al.* 2013). The only archaea detected in the anaerobic sludge at phylum level was *Euryarchaeota*, which took a tiny percentage of 0.65%. These results demonstrated that the AnMBR was running well under a hydrolysis acidification state. While in the MBR, *Proteobacteria* took a percentage of above 85%, followed by *Bacteroidetes*. These two phyla were widely reported to be dominant in MBRs (Gao *et al.* 2011; Ma *et al.* 2013). And a recent study (Liu *et al.* 2017) indicated that the phylum *Proteobacteria* was closely related to COD and NH_4^+-N removal under oxic conditions owing to nitrification.

Consumption of power and chemicals

Different alkaline liquor dosing strategies were adopted during the experimentation. During the first 10 days, no alkaline liquor was added into the MBR, and the pH value of the effluent decreased from 7.0 to 5.0 (Figure 4). Between days 11 and 41, potassium bicarbonate solution was continuously injected into the MBR. Meanwhile, the effluent pH dropped below 5.5 twice due to a pump malfunction and a PLC problem. To maintain a relatively steady effluent pH value, from day 42 on, the real time effluent pH values fed back by the online pH sensor were taken as set-points which controlled the alkaline

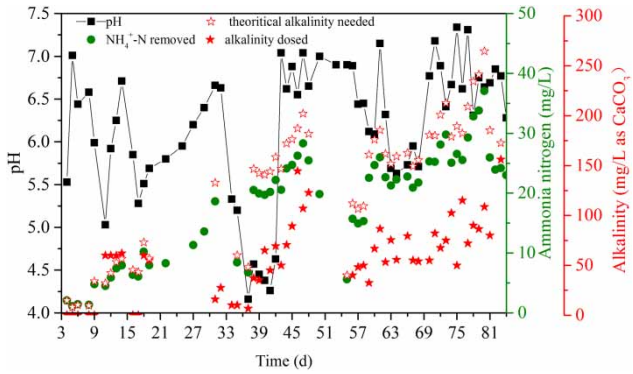


Figure 4 | The variation of alkaline liquor dosage and effluent pH values.

liquor dosing. When the alkaline diaphragm pump was set to start at the effluent pH value of 5.8 and stop at 5.9 the nitrification process kept going, but due to a lagging effect the effluent pH value could decrease to about 5.5, which was not appropriate for the higher plants' growth. Thus the final set-points were set at 6.5 and 7.0.

During the last 28 days, 2,818 L wastewater was treated in total and the consumption of potassium bicarbonate was 3,994 g, meaning 1.42 g potassium bicarbonate was needed to treat 1 L wastewater. This consumable need was much lower than that (20 g) of multifiltration and ion exchange beds on the ISS (Meyer *et al.* 2016).

In terms of power consumption, the running capacity of the WTRS was around 400 W, so the specific energy consumption was about 83 W·h/L H_2O. This value was higher than that (20 W·h/L H_2O) of the urine processor assembly on the ISS (Bobe *et al.* 2007). It is worth noting that devices onboard the ISS are all made-to-order products and they must have been optimized before they were installed. So, there is still space to decrease the power consumption of the WTRS.

CONCLUSIONS

The WTRS, which mainly combined an AnMBR and an MBR showed good performance treating synthetic terrestrial-based CELSS wastewater. The effluent could be used as qualified replenishment for a hydroponic system, with concentrations of COD, NH_4^+-N and NO_2^--N below 30 mg/L, 20 mg/L and 2 mg/L, respectively. Gas emission was also managed to meet the requirements of CELSS. The specific consumables consumption of 1.42 g/L H_2O was much lower than that of the multifiltration and ion exchange beds on the ISS, but the specific energy consumption of 83 W·h/L H_2O is higher than that of the urine processor assembly on the ISS. Results of microbial community analysis showed that the dominant phyla were *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* in the AnMBR while *Proteobacteria* and *Bacteroidetes* dominated in the MBR.

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REFERENCES

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association/American Water Works Association/Water Environmental Federation, Washington, DC, USA.

Bobe, L., Samsonov, N., Gavrilov, L., Novikov, V., Tomashpolskiy, M., Andreychuk, P., Protasov, N., Synjak, Y. & Skuratov, V. 2007 Regenerative water supply for an interplanetary space station: the experience gained on the space stations 'Salut', 'Mir', ISS and development prospects. *Acta Astronautica* **61** (1–6), 8–15.

Chen, L., Gu, Y., Cao, C., Zhang, J., Ng, J.-W. & Tang, C. 2014 Performance of a submerged anaerobic membrane bioreactor with forward osmosis membrane for low-strength wastewater treatment. *Water Research* **50**, 114–123.

Gao, D.-w., Fu, Y., Tao, Y., Li, X.-x., Xing, M., Gao, X.-h. & Ren, N.-q. 2011 Linking microbial community structure to membrane biofouling associated with varying dissolved oxygen concentrations. *Bioresource Technology* **102** (10), 5626–5633.

Grigoriev, A. I., Sinyak, Y. E., Samsonov, N. M., Bobe, L. S., Protasov, N. N. & Andreychuk, P. O. 2011 Regeneration of water at space stations. *Acta Astronautica* **68** (9–10), 1567–1573.

Judd, S. & Judd, C. 2011 *The MBR Book*. Elsevier Science, Oxford.

Liu, J., Zhang, H., Zhang, P., Wu, Y., Gou, X., Song, Y., Tian, Z. & Zeng, G. 2017 Two-stage anoxic/oxic combined membrane bioreactor system for landfill leachate treatment: pollutant

removal performances and microbial community. *Bioresource Technology* **243**, 738–746.

Ma, J., Wang, Z., Yang, Y., Mei, X. & Wu, Z. 2013 Correlating microbial community structure and composition with aeration intensity in submerged membrane bioreactors by 454 high-throughput pyrosequencing. *Water Research* **47** (2), 859–869.

Meyer, C. E., Pensinger, S., Adam, N., Pickering, K. D., Barta, D. & Shull, S. A. Results of the Alternative Water Process Test, a novel technology for exploration wastewater remediation. In: *46th International Conference on Environmental Systems*, Vienna, Austria, 10–14 July 2016.

Petrie, G. E. & Lupton, F. S. 1991 Performance characteristics of a low sludge bioreactor for wastewater treatment. *Waste Management & Research* **9** (5), 471–476.

Qin, L., Guo, S., Ai, W., Tang, Y., Cheng, Q. & Chen, G. 2013 Effect of salt stress on growth and physiology in amaranth and lettuce: implications for bioregenerative life support system. *Advances in Space Research* **51** (3), 476–482.

Salisbury, F. B., Gitelson, J. I. & Lisovsky, G. M. 1997 Bios-3: Siberian experiments in bioregenerative life support. *BioScience* **47** (9), 575–585.

Thomas, W., James, A., Neal, H., Roger, D. & David, G. 2011 Nonhazardous urine pretreatment method for future exploration systems. In: *41st International Conference on Environmental Systems*. American Institute of Aeronautics and Astronautics.

Xie, B., Zhu, G., Liu, B., Su, Q., Deng, S., Yang, L., Liu, G., Dong, C., Wang, M. & Liu, H. 2017 The water treatment and recycling in 105-day bioregenerative life support experiment in the Lunar Palace 1. *Acta Astronautica* **140**, 420–426.

Xu, Z. & Yao, M. 2013 Monitoring of bioaerosol inhalation risks in different environments using a six-stage Andersen sampler and the PCR-DGGE method. *Environmental Monitoring and Assessment* **185** (5), 3993–4003.

Yang, Q., Xiong, P., Ding, P., Chu, L. & Wang, J. 2015 Treatment of petrochemical wastewater by microaerobic hydrolysis and anoxic/oxic processes and analysis of bacterial diversity. *Bioresource Technology* **196**, 169–175.

Yu, D., Liu, J., Sui, Q. & Wei, Y. 2016 Biogas-pH automation control strategy for optimizing organic loading rate of anaerobic membrane bioreactor treating high COD wastewater. *Bioresource Technology* **203**, 62–70.

Zhang, J., Lv, C., Tong, J., Liu, J., Liu, J., Yu, D., Wang, Y., Chen, M. & Wei, Y. 2016a Optimization and microbial community analysis of anaerobic co-digestion of food waste and sewage sludge based on microwave pretreatment. *Bioresource Technology* **200**, 253–261.

Zhang, L., He, Y., Chen, M., Gao, M., Qu, T. & Wang, X. 2016b Pollution characteristics of antibiotic resistant bacteria from atmospheric environment of animal feeding operations. *Environmental Science* **37** (12), 4531–4537.

Zheng, X., Su, Y., Li, X., Xiao, N., Wang, D.-B. & Chen, Y. 2013 Pyrosequencing reveals the key microorganisms involved in sludge alkaline fermentation for efficient short-chain fatty acids production. *Enviromental Science & Technology* **47**, 4262–4268.

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